EFFECTS OF CHEMOSTERILANT FUMIGATION ON THE MORTALITY AND STERILITY OF BOLL WEEVILS

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EFFECTS OF CHEMOSTERILANT FUMIGATION ON THE MORTALITY AND STERILITY OF BOLL WEEVILS

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ABSTRACT

Laboratory experiments, using a simple closed-flask method and some highly active chemosterilants, were conducted to determine the effectiveness of fumigation as a method of sterilizing the boll weevil, *Anthonomus grandis* Boheman. After fumigation, males and females were separated, mated with normal, untreated boll weevils, and fed fresh diet pellets. A maximum 50 eggs were collected each week for 3 weeks after treatment. Half the eggs were checked daily for hatch, and half were implanted and observed 21 days for adult emergence. Fumigation was generally an effective sterilizing technique. The alkylating fumigants tepa, thiotepa, metepa, and *P,P*-bis(1-aziridinyl)-*N*-methylphosphinothioic amide (ENT-61585) were effective in sterilizing 92 to 100 percent of treated males for 3 weeks, and fecundity of treated females was reduced 88 to 100 percent. Tepa, thiotepa, and ENT-61585 were the most active compounds tested at 30° C and required only half the time of metepa or *P,P*-bis(1-aziridinyl)-*N*-methylphosphinic amide (ENT-51254) to sterilize boll weevils. The nonalkylating fumigants hempa and hexamethylphosphorothioic triamide (ENT-50918) were effective in reducing egg hatch, but recovery of fertility occurred 3 weeks after hempa treatment. None of the mixtures of the fumigant compounds was more effective as a sterilant than the individual compounds.

INTRODUCTION

In practically oriented experiments, chemosterilants have been applied to insects by dipping, spraying, or feeding but only to a limited extent by fumigation. Borkovec et al.[3] reported successful sterilization of the azuki bean weevil, a coleopterous insect, after 8.5 or more hours of fumigation; egg laying was significantly inhibited.

Fumigation as a method of sterilizing insects has many potential advantages. The equipment is relatively inexpensive, and only minimum amounts of sterilants are required. Large numbers of insects need not be maintained for extended periods, and it takes much less time to sterilize newly emerged, laboratory-reared insects by fumigation than by feeding a sterilant-treated diet. The technology for large-scale fumigation with insecticides is well developed and may be applied to chemosterilants with relatively minor modification.

Though more elaborate equipment may be required in large-scale experiments, a simple

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[3] Borkovec, A. B., Nagasawa, S., and Shinohara, H. 1968. Sterilization of the azuki bean weevil, *Callosobruchus chiensis*, by metepa and hempa. J. Econ. Entomol. 61: 695–698.

closed-flask method is adequate for small-scale laboratory application.[4] We report here the results of laboratory experiments on the sterilization of the boll weevil, *Anthonomus grandis* Boheman, with alkylating and nonalkylating chemosterilants applied by fumigation in a stationary atmosphere.

MATERIALS AND METHODS

The tests were conducted with an ebony strain of boll weevils developed and reared at Agricultural Research Service's Boll Weevil Research Laboratory at Mississippi State.[5] For each treatment, a small amount (0.25 g) of the candidate fumigant was dissolved in acetone and coated on the inside surface of a 200-ml Erlenmeyer flask that was left open for 24 hours to allow the acetone to evaporate. Then 25 pairs (occasionally 10 pairs) of boll weevils were placed in a small, stoppered, screen basket approximately 0.63 by 5.08 cm that was suspended from the bottom of the flask stopper. After the flask was sealed, it was fumigated for the desired time at 27.5°, 30°, or 33° C. Because the spiracles of anesthetized insects remain open, the boll weevils in some experiments were treated with CO_2 before fumigation to allow increased fumigant intake.

After fumigation, the males and females were separated, mated with normal, untreated boll weevils, and fed fresh diet pellets. A maximum 50 eggs were collected each week for 3 weeks after treatment. Half the eggs were checked daily for hatch (up to 7 days), and half were implanted and observed 21 days for adult F_1 emergence.

The alkylating agents used in this study were tepa, thiotepa, metepa, *P,P*-bis(1-aziridinyl)-*N*-methylphosphinothioic amide (ENT-61585), and *P,P*-bis (1-aziridinyl)-*N*-methylphosphinic amide (ENT-51254).[6] The nonalkylating agents used were hempa, hexamethylphosphorothioic triamide (ENT-50918), and *N,N,N',N'*-tetramethyl-*P*-morpholinophosphonic diamide (ENT-51008), and mixtures of ENT-61585 and metepa, hempa and ENT-50918, and ENT-51254 and ENT-51008.

[4] Chang, S. C., Borkovec, A. B., Woods, C. W., and Terry, P. H. 1973. Chemosterilization of male house flies by fumigation. J. Econ. Entomol. 66: 23–26.

[5] Bartlett, A. C. 1967. Genetic markers in the boll weevil. J. Hered. 58: 159–163. Gast, R. T., and Davich, T. B. 1966. Boll weevils. *In* C. N. Smith (ed.), Insect Colonization and Mass Production, pp. 406–418. Academic Press, New York.

[6] Arbitrary numbers are assigned to chemicals used in Agricultural Research Service entomological investigations. Prefix was recently changed from ENT to AI3 but the numbers remain the same.

RESULTS

Effects of Alkylating Fumigants

Fumigation of male and female boll weevils for 3 hours and males for 2 hours with tepa at 30° C resulted in nearly total sterility (table 1). The 3-hour tepa fumigation was hardly more effective than the 2-hour treatment in reducing egg hatch, but mortality was 60 percent more at 21 days after treatment. Fumigated for 3 hours, females laid a total of 16 eggs, 1 of which developed into an adult, whereas control females laid 138 eggs, 50 of which developed into adults.

A 3-hour fumigation of male and female boll weevils with thiotepa resulted in adult emergence of zero to 4.3 percent for 3 weeks after treatment compared to 34 to 38 percent in the control. Mortality of treated males was 28, 77, and 80 percent after 1, 2, and 3 weeks compared to 8, 15, and 28 percent in the control.

Fumigation for 6 hours at 33° C with metepa in a CO_2 atmosphere sterilized 92 to 100 percent of the males for 3 weeks, and treated females laid 7 sterile eggs.

Fumigation of male boll weevils with ENT-61585 for 3 and 4 hours resulted in 90 to 99 percent sterility (based on adult emergence) for 2 weeks, but sterility was only 75 to 85 percent 1 week later. Fumigation of females for 3 hours with ENT-61585 prevented egg laying. Fumigation of males for 4 hours with ENT-61585 in an atmosphere of CO_2 resulted in total sterility for 3 weeks. The cumulative mortality was 32, 45, and 80 percent for 1, 2, and 3 weeks, respectively.

ENT-51254 was 100 percent effective (based on adult emergence) as a male fumigant (5½-hour treatment) for 2 weeks, but 24 percent recovery occurred 1 week later; cumulative mortality of treated males was 0, 50, and 60 percent for 1, 2, and 3 weeks.

Effects of Nonalkylating Fumigants

A 5-hour fumigation with hempa in a CO_2

(Continued on page 5.)

TABLE 1.—*Results of chemosterilant fumigation of newly emerged boll weevils*[1]

Treatment				Results			
Fumigant	Replications	Hours fumigated	Sex[2]	Total eggs	Percent egg hatch	Percent adult emergence	Percent mortality, treated adults (cumulative for each treatment)
				1st week after treatment			
Alkylating agent:							
Tepa	2	2	M	50	24	0	5
Do	3	3	M	49	12	1	8
Do	2	3	F	4	100	25	20
Thiotepa	7	3	M	50	.7	0	28
Do	2	3	F	3	0	0	28
Metepa	1	6	M	50	4	8	0
Do	2	[3 4]6	M	50	4	4	30
Do	2	[3 4]6	F	6	50	0	50
ENT-61585	6	3	M	50	3	1	34
Do	2	3	F	0	0	0	35
Do	3	4	M	50	4	3	15
Do	2	[4]4	M	50	0	0	32
ENT-51254	4	4	M	45	51	10	5
Do	2	[5]5½	M[6]	50	0	0	0
Nonalkylating agent:							
Hempa	2	[4]5	M	30	12	7	37
Do	2	[4]5	F	1	100	100	30
Do	2	5½	M	50	0	0	40
Do	2	5½	F	15	20	20	30
ENT-50918	5	4	M	50	5	0	38
Do	2	[4]3	F	3	67	67	55
ENT-51008	4	8	M	40	70	23	6
Do	2	[4]8	M[6]	50	16	16	10
Do	2	[4]8	F[6]	50	72	22	12
Mixture:							
ENT-61585 + metepa	2	[5]2	M	50	16	4	7
Do	2	3	M[6]	50	4	0	33
Do	2	3	F[6]	1	0	0	49
Hempa + ENT-50918	2	5	M	50	4	2	5
Do	1	5	F	50	31	8	20
ENT-51254 + ENT-51008	2	2	M	50	78	14	7
Do	2	3	M	50	28	8	10
Control	26	...	..	50	74	36	8
				2d week after treatment			
Alkylating agent:							
Tepa	2	2	M	50	48	8	10
Do	3	3	M	50	12	3	62
Do	2	3	F	9	0	0	68
Thiotepa	7	3	M	49	3.6	.4	77
Do	2	3	F	1	0	0	84
Metepa	1	6	M	50	48	12	35
Do	2	[3 4]6	M	50	0	0	90
Do	2	[3 4]6	F	4	50	0	60

See footnotes at end of table.

TABLE 1.—*Results of chemosterilant fumigation of newly emerged boll weevils*[1]—Continued

Treatment				Results			
Fumigant	Replications	Hours fumigated	Sex[2]	Total eggs	Percent egg hatch	Percent adult emergence	Percent mortality, treated adults (cumulative for each treatment)
				2d week after treatment—Continued			
Alkylating agent—Continued:							
ENT-61585	6	3	M	36	12	10	83
Do	2	3	F	0	0	0	100
Do	3	4	M	50	8	8	62
Do	2	[4]4	M	49	0	0	45
ENT-51254	4	4	M	48	82	21	18
Do	2	[5]5½	M[6]	50	5	0	50
Nonalkylating agent:							
Hempa	2	[4]5	M	25	46	0	44
Do	2	[4]5	F	1	100	100	40
Do	2	5½	M	50	44	0	50
Do	2	5½	F	4	100	0	30
ENT-50918	5	4	M	40	20	7	62
Do	2	[4]3	F	5	100	40	70
ENT-51008	4	8	M	25	63	32	7
Do	2	[4]8	M[6]	50	36	16	10
Do	2	[4]8	F[6]	50	50	5	36
Mixture:							
ENT-61585 + metepa	2	[5]2	M	50	58	5	18
Do	2	3	M[6]	50	12	3	76
Do	2	3	F[6]	0	0	0	78
Hempa + ENT-50918	2	5	M	50	24	8	7
Do	1	5	F	40	32	6	40
ENT-51254 + ENT-51008	2	2	M	50	70	18	8
Do	2	3	M	50	50	12	48
Control	26	...	..	50	77	34	15
				3d week after treatment			
Alkylating agent:							
Tepa	2	2	M	50	76	([7])	10
Do	3	3	M	48	24	7	77
Do	2	3	F	3	0	0	72
Thiotepa	7	3	M	44	8.4	4.3	80
Do	2	3	F	0	0	0	88
Metepa	1	6	M	50	56	20	40
Do	2	[3 4]6	M	50	8	8	100
Do	2	[3 4]6	F	2	0	0	70
ENT-61585	6	3	M	13	0	25	85
Do	2	3	F	0	0	0	0
Do	3	4	M	29	23	15	65
Do	2	[4]4	M	33	0	0	80
ENT-51254	4	4	M	29	66	22	22
Do	2	[5]5½	M[6]	41	48	24	60

See footnotes at end of table.

TABLE 1.—*Results of chemosterilant fumigation of newly emerged boll weevils*[1]—Continued

Fumigant	Treatment: Replications	Treatment: Hours fumigated	Sex[2]	Results: Total eggs	Results: Percent egg hatch	Results: Percent adult emergence	Results: Percent mortality, treated adults (cumulative for each treatment)
				3d week after treatment—Continued			
Nonalkylating agent:							
Hempa	2	[5]	M	14	100	33	44
Do	2	[5]	F	4	0	0	40
Do	2	5½	M	50	46	32	50
Do	2	5½	F	12	37	22	40
ENT-50918	5	4	M	28	21	7	72
Do	2	[3]	F	4	100	50	70
ENT-51008	4	8	M	27	56	18	10
Do	2	[8]	M[6]	50	43	12	30
Do	2	[8]	F[6]	28	47	16	40
Mixture:							
ENT-61585 + metepa	2	[2]	M	50	70	29	20
Do	2	3	M[6]	50	4	4	80
Do	2	3	F[6]	0	0	0	80
Hempa + ENT-50918	2	5	M	50	56	10	28
Do	1	5	F	24	63	40	44
ENT-51254 + ENT-51008	2	2	M	50	80	18	8
Do	2	3	M	50	35	11	53
Control	26	...	..	38	70	38	28

[1] Fumigation at 30° C except as indicated in footnotes 3 and 5. 25 pairs boll weevils used per test except as indicated in footnote 6.

[2] M, treated males mated to normal females. F, treated females mated to normal males.

[3] Fumigation at 33° C.

[4] Fumigation in an atmosphere of CO_2.

[5] Fumigation at 27.5° C.

[6] 10 pairs boll weevils used in 1 replication only.

[7] Plate discarded because of contamination.

atmosphere reduced adult male emergence from percent the first week to zero the second, but emergence was 33 percent after 21 days. Females from the same treatment produced 2 adults from 6 eggs compared with 50 adults from 138 eggs in the control. A 5½-hour fumigation without CO_2 was comparable to the 5-hour treatment with CO_2, and mortality ranged from 30 to 50 percent for the 3-week period.

Females from a 3-hour fumigation with ENT-50918 in a CO_2 atmosphere laid only 12 eggs. A 4-hour fumigation of males with ENT-50918 resulted in zero to 7 percent adult emergence

treatments was [illegible]

and 62 to 72 percent the following 2 weeks.

An 8-hour fumigation of boll weevils with ENT-51008 in a CO_2 atmosphere sterilized 84 to 88 percent of the males and 78 to 95 percent of the females for 3 weeks.

Effects of Mixtures

A 3-hour fumigation of males and females with ENT-61585 + metepa resulted in adult emergence of zero to 4 percent for 3 weeks, and

treated females produced only 1 infertile egg in 3 weeks. Mortality averaged 41, 77, and 80 percent after 1, 2, and 3 weeks compared with 8, 15, and 28 percent in the control.

A 5-hour fumigation of males with hempa + ENT-50918 resulted in 90 to 98 percent sterility for 3 weeks, and mortality was no higher than in the control. Fumigation of females for 5 hours reduced adult emergence to 6 to 8 percent for 2 weeks, but 40 percent emergence occurred after 3 weeks. Fecundity of treated females was slightly less than in the control throughout the treatment.

A 3-hour fumigation of males with ENT-51254 + ENT-51008 resulted in 8 to 12 percent adult emergence for 3 weeks compared with 34 to 38 percent emergence in the control. Mortality was only 10 percent after 1 week but was 53 percent after 3 weeks.

DISCUSSION

In these laboratory experiments, fumigation of newly emerged boll weevils with chemosterilants was an effective sterilization technique. The alkylating fumigants tepa, thiotepa, metepa, and ENT-61585 sterilized 92 to 100 percent of treated males for 3 weeks, and fecundity of treated females was reduced 88 to 100 percent. Tepa, thiotepa, and ENT-61585 were the most active fumigant compounds tested at 30° C; these treatments required only half the time of metepa or ENT-51254 to sterilize boll weevils.

The nonalkylating fumigants hempa and ENT-50918 proved to be as effective as the alkylating fumigants in reducing egg hatch or fecundity; however, recovery of fertility occurred in the third week after hempa treatment.

None of the mixtures of the candidate fumigants proved to be more effective in sterilizing boll weevils than the individual compounds. However, the mixture ENT-61585 + metepa sterilized 96 to 100 percent of the males in 1 to 3 hours less than the individual compounds.

In order of decreasing activity, the chemosterilants ranked as follows: ENT-61585, thiotepa, ENT-61585 + metepa, tepa, metepa, ENT-50918, hempa + ENT-50918, ENT-51254, hempa, ENT-51254 + ENT-51008, and ENT-51008. In order of decreasing toxicity, the ranking was metepa; ENT-61585, thiotepa, and ENT-61585 + metepa; tepa; ENT-50918; ENT-51254; ENT-51254 + ENT-51008; hempa; ENT-51008; and hempa + ENT-50918.

* 1976-G.P.O./1750-S/671-583/75